

Materials List for:

Visualization, Quantification, and Mapping of Immune Cell Populations in the Tumor Microenvironment

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Materials

Name	Company	Catalog Number	Comments
Antigen Retrieval Solution: Sodium Citrate Buffer (10 mM Sodium Citrate, 0.05% v/v Tween 20, pH 6.0)			
Blocking Solution: 1 % BSA, 10 % filtered human serum, 10 % filtered donkey serum, 0.1 % Tween 20, and 0.3% Triton in PBS			
Bovine serum albumin (BSA)	Multicell	800-095-EG	
Coplin jars (EASYDIP SLIDE STAINING SYSTEM)	Newcomersupply	5300KIT	
Cover slides	Fisherbrand	12-545E 22*50	
Direct Red 80	Sigma Aldrich	365548	
Donkey Serum	Sigma Aldrich	D9663	
Ethanol 100%			
Electric pressure cooker	Salton		
Eosin	Leica Biosystems	3801600	CAUTION, eye irritation
Fast Green FCF	Sigma Aldrich	F7252	CAUTION, harmful by inhalation, ingestion and skin absorption
FFPE section (4µm) slides			
Glycine 0,1 M in PBS			
Hematoxylin Stain Solution, Gil 1. Formulation, Regular Strength	Ricca Chemical Company	3535-32	
Holder (EasyDip Staining Jar Holder)	Newcomersupply	5300RK	
Human Serum	Gemini	22210	
Humidity chamber	Millipore Sigma	Z670138-1EA	
Pap pen	abcam	ab2601	
PBS			
PBS-Tween 20 (0.1% v/v)			
Permount Mounting Media	Fisher Chemical	SP15-500	
Picric Acid 1.3 %	Sigma Aldrich	P6744	CAUTION, skin and eye irritation

Picro-Sirius Red/Fast Green solution: Fast Green 0.1 % w/v + Sirius Red 0.2 % w/v in 1,3 % picric acid solution			
Primary Antibody Anti- α SMA	Mouse IgG2a 1A4	Sigma A2547	Dilution 1/100
Primary Antibody Anti-CD34	Mouse IgG1 HPCA1/763	Novus Biologicals NBP2-44568	Dilution 1/250
Primary Antibody Anti-Cytokeratin 8/18	Rabbit EP17/EP30	Agilent IR09461-2	Ready to use
Primary Antibody Anti-CD68	Mouse KP1	Abcam ab955	Dilution 1/200
Primary Antibody Anti-Desmin	Rabbit Polyclonal	Invitrogen PA5-16705	Dilution 1/200
Primary Antibody Anti-CD4	Mouse N1UG0	Affymetrix 14-2444	Dilution 1/250
Primary Antibody Anti-FoxP3	Rabbit 1054C	R & D MAB8214	Dilution 1/100
Primary Antibody Anti-MPO	Goat Polyclonal	R & D Systems AF3667	Dilution 1/250
Primary Antibody Anti-CD3	Rabbit SP7	Abcam ab16669	Dilution 1/200
Primary Antibody Anti-CD8	Mouse C8/144B	Invitrogen 14-0085-80	Dilution 1/200
Secondary Antibody Donkey anti-mouse A488	Polyclonal	Invitrogen A-21202	Dilution 1/500
Secondary Antibody Donkey anti-Rabbit A488	Polyclonal	Invitrogen A-21206	Dilution 1/500
Secondary Antibody Donkey anti-goat A568	Polyclonal	Invitrogen A-11057	Dilution 1/500
Secondary Antibody Donkey anti-rabbit A647	Polyclonal	Invitrogen A-31573	Dilution 1/500
Secondary Antibody Rat anti-mouse IgG1 A647	RMG1-1	Biologend 406618	Dilution 1/500
Secondary Antibody Goat anti-mouse IgG2a CF568	Polyclonal	Sigma Aldrich SAB4600315	Dilution 1/500
Secondary Antibody Donkey anti-mouse DyLight 755	Polyclonal	Invitrogen SA5-10171	Dilution 1/500
Secondary Antibody Donkey anti-rabbit DyLight 755	Polyclonal	Invitrogen SA5-10043	Dilution 1/500
SDS	BioShop	SDS001,500	CAUTION, oral skin and eye toxicity
Shandon multi-program robotic slide stainer	LabX	11384903	
Shandon Xylene Substitute,	Thermo Fisher Scientific	CA89413-336	CAUTION, Flammable, skin and eye irritation, Harmful when inhaled
Shaking water bath			
SlowFade Gold antifade reagent with DAPI	Invitrogen	S36938	
Sodium Citrate Dihydrate	Millipore Sigma	1545801	CAUTION, eye irritation
Stripping Buffer: mix 20 ml 10% w/v SDS with 12.5 ml 0.5 M Tris-HCl (pH 6.8), and 67.5 ml ultra-pure water. Under a fume hood, add 800 μ L of 2-mercapto ethanol (114,4 mM final concentration)			
Triton X-100	Sigma Aldrich	T8787-50ML	
Tris-HCl	BioShop	77-86-1	
Tween 20	Fisher Scientific	BP337-500	
VIS Software	Visiopharm		
Whole slide scanner Olympus BX61VS	Olympus		Microscope: Olympus Slide Scanner BX61VS, 5 slides scanner, motorized stage,

			autofocus. Camera: Lightsource: Xcite-120. Filters: BrightLine® Sedat filter set (# LED-DA/FI/TR/Cy5-4X4M-B-000, Semrock)
Xylene	Sigma Aldrich	214736-4L	CAUTION, Flammable, skin and eye irritation, Harmful when inhaled
Xylene : Ethanol solution (1:1 v/v)			
2-mercaptoethanol	Sigma	M6250	CAUTION, harmful by ingestion, inhalation, fatal if sking absortion. Eye irritation. Use fume hood